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STANDARDIZATION OF SEED TESTING PROTOCOL IN GRAIN AMARANTH (*Amaranthus hypochondriacus* L.)

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ABSTRACT

A laboratory experiment was conducted at Department of Seed Science and Technology, College of Agriculture, UAS, GKVK, Bengaluru. Grain amaranth seeds were subjected to germination at different substrates (sand, between paper and top of paper) and temperatures (20, 25, 30, 20/25 and 25/30 °C). Among the substrates, higher seed germination (80.06 %), mean seedling length (8.90 cm), mean seedling dry weight (4.25 mg), speed of germination (31.40) and seed vigour index-I and II (720 and 344) were recorded in between paper method. Among the temperatures, higher germination (86.55 %), mean seedling length (10.20 cm), mean seedling dry weight (4.96 mg) and seed vigour index-I and II (884 and 430) were recorded at alternative temperature of 25/30 °C.

***Key words*:** Seed quality, substrate, temperature, seed testing.

Introduction

Grain amaranths belong to Amaranthaceae family and are herbaceous annual crop plants. They grow rapidly in a wide range of agroecological environments ranging from tropics to semi-arid and arid areas (Alemayehu *et al*., 2015). Grain amaranth is a promising pseudo cereal crop to feed the human population (Mekonnen *et al*., 2018) and a good forage for livestock (Mustafa *et al*., 2011; Ahrar *et al*., 2020). It has pale seeds and is known for its high nutritional value, being particularly rich in lysine (5%) and sulphur amino acids (4.4%). These amino acids are essential for a well-balanced human diet and are typically limited in the plant kingdom. Grain amaranth is often referred to as the “Poor man’s spinach” due to its affordability and is recommended as a means to combat malnutrition in various parts of the world. Grain amaranth surpasses cereal crops in terms of protein quality, primarily due to its high content of essential amino acids, including lysine, methionine and cysteine. Its significance lies in the excellent quality of its leaves and tender stems as a food source, along with its high biocompatibility, nutritional value, antioxidants, mineral content, and cost-effectiveness compared to cereal crops (FAO, 2020). The nutritional attributes of grain amaranth, such as fiber and iron content, as well as its low levels of saturated fats and gluten-free nature, became widely recognized. Consequently, the crop has gained importance and experienced a resurgence as a health-conscious crop in numerous countries, including India.

One notable advantage of grain amaranth is its possession of the C4 pathway, which allows for efficient photosynthesis rates. This crop exhibits remarkable adaptability, capable of thriving in harsh environments. The genus amaranth is characterized by dibasic chromosomes, with a distribution nearly equal across the amaranth section and a chromosomal makeup of (x = 16, 17). Grant (1959) has documented the chromosome numbers of 30 Amaranthus species. Among these, four species have 2n = 32 chromosomes, while the remaining species have 2n = 34. Within the 30 species, *Amaranthus*

Table 1: Effect of substrate and temperature on seed germination and mean seedling length of grain amaranth.

Treatments	Seed germination (%)						Mean seedling length (cm)					
	T_1	T_2	T_3	T_4	T_5	Mean	T_1	T_2	T_3	T_4	T_5	Mean
S_1	63.33	81.33	72.33	77.33	84.67	75.80	7.00	8.93	7.80	8.40	9.77	8.38
S_2	71.67	83.33	75.33	80.33	89.67	80.06	7.50	9.53	8.13	8.73	10.60	8.90
S_3	69.33	82.33	73.33	79.33	85.33	77.93	7.33	9.30	8.00	8.60	10.23	8.69
Mean	68.11	82.33	73.66	79.00	86.55		7.27	9.25	7.97	8.57	10.20	
	S		T		S×T		S		T		S×T	
SEm±	0.61		0.78		1.36		0.05		0.06		0.11	
CD (P=0.05)	1.76		2.27		NS		0.14		0.18		NS	
CV (%)	3.03						2.23					

*NS: Non-significant, S_1: Top of paper (TP), S_2: Between paper (BP), S_3: Sand, T_1: 20°C, T_2: 25°C, T_3: 30°C, T_4: 20/25°C and T_5: 25/30°C

caudatus L. (2n = 32), *Amaranthus cruentus* L. (2n = 34), and *Amaranthus hypochondriacus* L. (2n = 32) are domesticated species. Among these three, *Amaranthus caudatus* L. exhibits a drooping growth habit, while the other two species are erect.

The use of quality seed is important for successful crop production. Therefore, it is crucial to increase the seed production of grain amaranth to meet growing demand. The quality of the seed lot is primarily evaluated through a seed testing procedure. Germination test has been accepted as an index of seed quality, which depends on the intrinsic characteristics of seeds and environmental factors such as temperature and substrate. Temperature affects the rate of imbibition and chemical reactions during germination process which influences uniformity and overall germination rate. Some species respond better at constant temperature while others best germinate at alternate temperature. The germination medium used plays a crucial role in the germination process of seeds, as it must meet specific requirements such as substrate to be non-toxic, free from moulds, and provide sufficient aeration and moisture for the seeds to germinate successfully. Before recommending a particular temperature and medium for adoption, it is necessary to standardize and determine the optimal temperature and

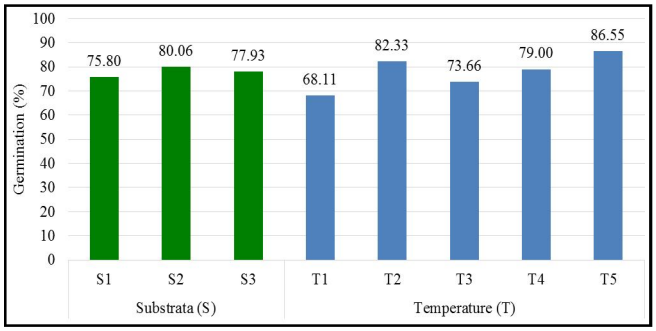

Fig. 1: Effect of substrate and temperature on seed germination of grain amaranth. [S1: Top of paper (TP), S2: Between paper (BP), S3: Sand, T1: 20°C, T2: 25°C, T3: 30°C, T4: 20/25°C and T5: 25/30°C]

medium for testing the germination potential of grain amaranth. Currently no standardized seed testing protocol have been developed for grain amaranth (*Amarathus hypochondriacus* L.). Therefore, the current study aims at developing methodology to evaluate germination and to establish a standard for routine in grain amaranth seed quality assessment.

Material and Methods

The present investigation was carried out in the Department of Seed Science and Technology, University of Agricultural Sciences, GKVK, Bengaluru. The seeds of grain amaranth variety KBGA-15 were collected from AICRP on Potential Crop, UAS, GKVK, Bengaluru. For seed germination testing protocol, standard growing media *viz.*, top of paper, between paper and sand were used and temperature *viz.*, constant temperature of 20°C, 25°C and 30°C and alternative temperature of 20/25°C and 25/30°C were employed in the experiment for standardization of substrate and temperature regime. The seeds were put to germination in top of paper by using blotters, between paper and trays containing sand. The germination test was carried out in walk in seed germinator. For alternate temperature, the lower temperature level was maintained for 16 hours and the higher temperature for 8 hours in a cycle of 24 hours. Seed quality parameters *viz.*, seed germination, mean seedling length, mean seedling dry weight, speed of germination, seed vigour index-I and II were evaluated.

Results and Discussion

Seed germination (%)

The data on seed germination (%) as influenced by different media with different temperatures is presented in Table 1 and Fig. 1. The seed germination percentage was significantly higher (80.06 %) in between paper method (S_2), followed by top of paper method (S_3) (77.93 %) and lower seed germination (75.80 %) was recorded in sand method (S_1). Higher seed germination obtained

Table 2: Effect of substrate and temperature on mean seedling dry weight and speed of germination of grain amaranth.

Treatments	Mean seedling dry weight (mg)						Speed of germination					
	T_1	T_2	T_3	T_4	T_5	Mean	T_1	T_2	T_3	T_4	T_5	Mean
S_1	3.17	4.33	3.60	3.90	4.70	3.94	17.03	27.01	37.81	22.66	34.16	27.73
S_2	3.50	4.53	3.80	4.23	5.20	4.25	21.51	33.39	40.45	25.54	36.16	31.40
S_3	3.37	4.40	3.77	4.13	5.00	4.13	19.52	30.45	38.59	24.65	35.67	29.77
Mean	3.34	4.42	3.72	4.08	4.96		19.35	30.28	38.94	24.28	35.32	
	S		T		S×T		S		T		S×T	
SEm±	0.02		0.03		0.33		0.32		0.42		0.73	
CD (P=0.05)	0.07		0.10		NS		0.94		1.22		NS	
CV (%)	2.53						4.29					

*NS: Non-significant, S_1: Top of paper (TP), S_2: Between paper (BP), S_3: Sand, T_1: 20°C, T_2: 25°C, T_3: 30°C, T_4: 20/25°C and T_5: 25/30°C

in BP method may be due to continuous and adequate moisture supply which might have led to proper imbibition. These results were similar to the findings of Gowda *et al.*, (2012) in *Cassia angustifolia* and Alves *et al.*, (2015) in guava seeds. Seeds tested in alternating temperature of 25/30 °C (T_5) recorded significantly higher seed germination (86.55 %) followed by temperature of 25 °C (T_2) (82.33 %) and lowest seed germination (68.11 %) was recorded in T1 (20 °C). The variations in temperature could cause activation of enzymes relating to seed germination. Poor and delayed seed germination at 20 °C might be due to reduced biochemical activity that might have led to slow seed germination.

Mean seedling length (cm)

Different substrate had a significant impact on mean seedling length of grain amaranth. Maximum mean seedling length (8.90 cm) was recorded in between paper method (S_2) followed by top paper method (S_3) (8.69 cm) and lower mean seedling length (8.38 cm) was recorded in sand method (S_1) (Table 1). Mean seedling length of grain amaranth was Significantly influenced by different temperature. Seeds tested in alternating temperature of 25/30 °C (T_5) recorded higher mean seedling length (10.20 cm) followed by temperature of 25 °C (T_2) (9.25 cm) and lower mean seedling length (7.27 cm) was recorded in T_1 (20 °C). Increase in temperature enhances the metabolic activity which in turns improves germination process and also increases the growth of the embryo and leads to the maximum seedling length. Results of Muhl *et al.*, (2011) stated that alternate temperature trigger the seedling emergence and seedling growth and accumulation of seedling dry weight and findings of Oliveira *et al.*, (2015) for *Callisthene fasciculata* Mart. showed better development of seedlings on between paper method.

Mean seedling dry weight (mg)

The data on mean seedling dry weight (mg) as influenced by different media with different temperatures is presented in Table 2. Mean seedling dry weight (4.25 mg) was recorded significantly higher in between paper method (S_2) followed by top of paper method (S_3) (4.13 mg) and lower mean seedling dry weight (3.94 mg) was recorded in sand method (S_1). Findings of Alves *et al.*, (2002) showed that substrate between paper had highest values for dry weight for *Mimosa caesalpiniifolia*. Seeds tested in alternating temperature of 25/30 °C (T_5) recorded significantly higher mean seedling dry weight (4.96 mg) followed by temperature of 25 °C (T_2) (4.42

Table 3: Effect of substrate and temperature on seed vigour index-I and II of grain amaranth.

Treatments	Seed vigour index - I						Seed vigour index - II					
	T_1	T_2	T_3	T_4	T_5	Mean	T_1	T_2	T_3	T_4	T_5	Mean
S_1	444	727	564	650	827	542	201	353	261	302	398	303
S_2	538	795	613	702	951	720	251	378	286	340	466	344
S_3	509	766	587	683	873	684	234	362	276	328	427	325
Mean	497	763	588	678	884		228	364	274	323	430	
	S		T		S×T		S		T		S×T	
SEm±	8.62		11.13		19.29		4.14		5.35		9.27	
CD (P=0.05)	24.91		32.17		NS		11.98		15.46		NS	
CV (%)	4.90						4.95					

*NS: Non-significant, S_1: Top of paper (TP), S_2: Between paper (BP), S_3: Sand, T_1: 20°C, T_2: 25°C, T_3: 30°C, T_4: 20/25°C and T_5: 25/30°C

mg) and lower mean seedling dry weight (3.34 mg) was recorded in seeds tested under temperature of 20 °C (T_1). Lima *et al.*, (2011) concluded that alternate temperature 20/30 °C and 20/35 °C provided good dry mass of seedling for *Caesalpinia pyramidalis* tul seeds.

Speed of germination

The data on speed of germination as influenced by different media with different temperatures is presented in Table 2. Significantly maximum speed of germination (31.40) was recorded in between paper method (S_2) followed by top of paper method (S_3) (29.77) and lower speed of germination (27.73) was recorded in sand method (S_1). Seeds tested in temperature of 30 °C (T_3) recorded significantly higher speed of germination (38.94) followed by alternating temperature of 25/30 °C (T_5) (35.32) and lower speed of germination (19.35) was recorded in seeds tested under temperature of T_1 (20 °C). When seeds were exposed to low temperature (20 °C), in general, there was a delay in germination from reduced metabolic activities. On the contrary, when seeds were placed in between paper at high temperature (30 °C) seeds germinated more rapidly as a result of protein denaturation coming from increased metabolic activities. This result was similar to the findings of De Jesus *et al.*, (2017) in quinoa seeds.

Seed Vigour Index-I

The data on seed vigour index-I as influenced by different media with different temperatures is presented in Table 3. Significantly higher seed vigour index-I (720) was recorded in between paper method (S_2) followed by top of paper method (S_3) (684) and lower seed vigour index-I (542) was recorded in sand method (S_1). Seeds tested in alternating temperature of 25/30 °C (T_5) recorded significantly higher seed vigour index-I (884) followed by temperature of 25 °C (T_2) (763) and lower seed vigour index-I (497) was recorded in 20 °C (T_1). This result was similar to the findings Buriro *et al.*, (2011) in wheat seed.

Seed vigour index-II

The data on seed vigour index-II as influenced by different media with different temperatures is presented in Table 3. Significantly maximum seed vigour index-II (344) was recorded in between paper method (S_2) followed by top of paper method (S_3) (325) and minimum (303) was recorded in sand method (S_1). Seeds tested in alternating temperature of 25/30 °C (T_5) recorded significantly higher seed vigour index-II (430) followed by temperature of 25 °C (T_2) (364) and less (228) was recorded in T_1 (20 °C). Seed vigour indices are sign of fast germination and speed of growth. These results are in lines with the results of Geetha (2011) for *Cenchrus glaucus*.

Conclusion

The experimental results revealed that both substrate and temperature significantly influenced the seed germination and seedling performance in grain amaranth. Amongst the substrate, the between-paper (BP) method recorded the superior performance in seed germination, mean seedling length, mean seedling dry weight, speed of germination, seed vigour index-I, and seed vigour index-II. Amongst the temperatures, germination and seedling performance were superior under the alternating temperature of 25/30°C, indicating that this temperature regime provides the most favourable conditions for seed germination and subsequent seedling growth. Therefore, it is concluded that the germination test of grain amaranth could be conducted at an alternative temperature of 25/30°C using between paper as a substratum.

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